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THE ETIOLOGY OF DIPHTHERIA.

By PROFESSOR WILLIAM H. WELCH, M. D., and
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In 1884 Löffler, in an article published in the second volume of the *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, concluded from the bacteriological examination of a large number of cases of diphtheria that a species of bacillus found by him frequently in diphtheritic membranes and carefully studied in its morphology and biology, and identified with the one described the previous year by Klebs, is the only one present which can be supposed to be of pathogenetic importance. He expressed himself, however, for several reasons with commendable reserve as to the etiological significance of this bacillus. Subsequent researches of a large number of investigators have removed one by one the doubts which Löffler had expressed until at the present time nearly all bacteriologists are convinced that the Klebs-Löffler bacillus is the specific cause of primary diphtheria. As the results reached by these various investigators have been summarized not long since by Löffler,² it is not necessary to describe them here in detail. It will suffice to mention the names of Babes, d'Espine, v. Hoffmann, Ortmann, Roux and Yersin, Kolisko and Paltauf, Zarniko, Sörensen, Escherich, Klein. Since the publication of Löffler's critical review in 1890, the most important contributions have been the investigations of the toxic albumens produced in cultures of the diphtheritic bacillus by Fränkel and Brieger, and the experimental production of immunity against inoculation with the diphtheritic bacilli or their poisonous products by Fränkel and by Behring.³

¹ The results of the investigations reported in this paper were communicated to the Johns Hopkins Hospital Medical Society at the meetings held June 2nd, 1890, and January 9th, 1891.

² Löffler. *Der gegenwärtige Stand der Frage nach der Entstehung der Diphtherie. Deutsche Med. Wochenschrift*, 1890, Nos. 5 and 6.

³ Fränkel and Brieger, *Berliner Klin. Wochenschrift*, 1890, Nos. 11 and 12. Fränkel, *Berliner Klin. Wochenschrift*, 1890, No. 49. Behring, *Deutsche Med. Wochenschrift*, 1890, No. 50.



By these investigations it has been determined that the Klebs-Löffler bacillus is present in all cases of primary diphtheria in the diphtheritic deposits, that it does not invade the blood or organs or even the mucous membrane affected, that in susceptible animals the disease can be reproduced in all its features, even to the production of paralysis, that the constitutional symptoms, the paralysis and the changes peculiar to diphtheria in internal organs are caused by a toxic albuminous substance or substances which have been separated in a condition approaching purity and which are produced by the local action of the bacilli in inoculated animals as well as in cultures, and that by various methods a greater or less degree of immunity can be artificially conferred upon susceptible animals and that under certain conditions susceptible animals can be cured after inoculation with virulent cultures.

In view of these convincing results it might be thought unnecessary to publish any further observations upon the bacteriological examination of cases of diphtheria; but Löffler, in the critical review mentioned, has called attention to the apparently discordant observations which have been made in this country by Prudden. In 24 cases of diphtheria in children examined by Prudden¹ he failed to find in a single instance, either by microscopical examination of the membranes or by cultures, the Klebs-Löffler bacillus. Recognizing the thorough competence of Prudden as a bacteriologist, Löffler says, "I do not believe that in North America a form of diphtheria prevails different from that with us. With us the bacilli are found regularly by every investigator, there they are constantly missed. Further investigations must and will clear up this contradiction." And he concludes his article with the sentence, "A simple 'Liquet' can not be affirmed until investigations inconsistent with this, such as those of Prudden in North America, have been shown to be erroneous."

We consider that the excellence of his methods, his well known thoroughness and accuracy and his well deserved reputation as a bacteriologist, give to the results published by Prudden the greatest weight. Nor do we regard it necessary to demonstrate that his observations are erroneous as suggested by Löffler. In the estimation of his results the following points mentioned in his article ought to be considered. All but two of the twenty four cases occurred in children's asylums or hospitals, 16 being in a

¹ Prudden. *Am. Jour. of the Med. Sciences*, April and May, 1889.

large foundling asylum in which measles and scarlatina were prevalent. In 7 cases the diphtheria complicated or was preceded by measles, in 3 by scarlatina, in 1 by whooping cough, in 4 by phlegmonous or erysipelalous inflammation, and in only 9 was the diphtheria apparently uncomplicated by notable previous disease; 4 cases were in children under one year of age, one of these being 6 days and another 7 days old. Of the apparently uncomplicated cases it is not clear from the article how many developed in the hospital under the same epidemic influences as the majority, but that some did is expressly stated.

It seems to us, therefore, clear that the cases examined by Dr. Prudden, taken as a series, should not be regarded as cases of primary diphtheria, but rather as diphtheria secondary to scarlatina, measles, erysipelas, or as developing in a situation where these diseases prevailed. While we do not possess all of the information desirable as to the etiology of the pseudo-membranous anginas secondary to various infectious diseases, and while we know that some of these are etiologically identical with primary diphtheria, it is certain that many of these cases should be separated from primary diphtheria. It has been shown by Wurtz and Bourges,¹ as well as by others, that the pseudo-membranous angina, occurring in the early stages of scarlatina, is accompanied regularly by a streptococcus probably identical with that found by Prudden, and not by the Klebs-Löffler bacillus. While it seems remarkable that in not a single instance, even of the apparently primary cases of diphtheria, did Prudden find the Löffler bacillus, we consider, for the reasons stated, that his observations need not be interpreted as contradictory to the conclusions of others who have studied cases of primary diphtheria. Nevertheless, it is evidently desirable that further bacteriological examination of cases of diphtheria occurring in this country should be made—the more so in view of the statements of Löffler already quoted.

In May, 1890, we therefore began the bacteriological examination of the membranous exudations in cases of diphtheria occurring in Baltimore. At the time of our examinations diphtheria was not prevailing as an epidemic, and we were unable to secure a large number of cases. In consequence, however, of the uniformity of the results obtained, and of their correspondence with those of European investigators in hundreds of cases, it did not seem necessary to examine a very large number in order to establish the etiological identity of our diphtheria with that in other countries.

We have, however, waited before publishing our results in order to obtain more cases, but as we have not been fortunate in this respect, we have decided not to wait longer for additional cases.

In all of the cases of diphtheria examined by us the clinical diagnosis was positive, and all were primary diphtheria developing in healthy children. All of the cases were in private practice and occurred in different parts of the city. For the opportunity of examining the cases we are indebted to Drs. Jay, Cordell, Lockwood, Booker, McCormick and Neff. The examinations were all made with bits of membrane removed during life. At first we removed these bits from the tonsils, or soft palate or pharynx, by means of sterilized forceps, but we afterward found it sufficient, as a rule, to touch or scrape or slightly bore into diphtheritic exudations in these situations with a sterilized platinum wire loop which had been flattened. Small particles of diphtheritic membrane, thus removed, were transferred to a sterilized test-tube, and carried to the laboratory where they were at once examined.

In each case cover-glass preparations were made from the membranous particles, and then, usually after washing in sterilized salt solution, a series of smear cultures was made in five or six tubes of slanting glycerine agar, or of Löffler's blood-serum and bouillon mixture, and a series of three or four plate cultures with glycerine agar (Petri's dishes being used) was prepared. When the material sufficed we likewise often made similar cultures in nutrient gelatine or plain nutrient agar. Only in the last two cases which we examined did we make use of the blood-serum and sugar bouillon mixture recommended by Löffler, upon which we found the diphtheritic bacillus to grow much more rapidly and luxuriantly than upon any of the other media used, but as at first we did not have this medium prepared, and as we nevertheless had no difficulty in obtaining positive results with the other media, particularly the glycerine agar, we continued to use the latter in most of our work. There are, however, distinct advantages in the use of the blood-serum bouillon medium, upon which the colonies of the Klebs-Löffler bacillus at a temperature of 35° C. are readily recognized in 18 to 24 hours. The study of the colonies we found a decided help in the isolation of the diphtheritic bacillus, and their characteristic features are well marked in glycerine-agar plate cultures, which, therefore, may be recommended as a most useful medium for isolating the organism.

After separating the bacillus in pure culture, its virulence was tested in each case by inoculation of guinea-pigs. We had for comparison a pure culture of the Löffler bacillus, obtained from the Hygienic Institute in Berlin, for which we are indebted to Dr. von Esmarch. The identification of the bacillus, in our cases, was based therefore upon the study of its morphology, its behavior in various culture media, and its effects when inoculated into guinea-pigs and other animals. In all respects we found it identical with the Löffler bacillus in the culture obtained from Berlin.

The following is a brief summary of the cases examined :

CASE 1.—Negro girl, 7 years old, has been sick two days. A thick whitish membrane covers tonsils, pharynx and anterior surface of uvula and soft palate. The child recovered. A piece of membrane 2 mm. thick and 5 mm. long was removed with forceps. Microscopical examination showed the usual structure of a diphtheritic membrane. Cover-glass preparations showed, singly and in clumps, bacilli averaging about the length of tubercle bacilli but thicker, often with one, sometimes with both ends swollen, frequently in one or both ends a small round body staining more deeply with methylene blue than the rest of the bacillus. These bacilli sometimes appeared slightly curved or broken into irregular segments. In addition, in considerable number, were found cocci single, in pairs and occasionally in chains, and a few long, slender bacilli were present.

Glycerine agar plate cultures showed in No. 1 diffuse cloudiness from abundant growth, in No. 2 very numerous scattered small colonies, and in Nos. 3 and 4 well distributed colonies. With the exception of 4 large white colonies of a coarse coccus in No. 1, only colonies of a streptococcus and of the Löffler bacillus were recognized, these two being in about equal number. The surface colonies of the Löffler bacillus were readily recognized in cultures two or three days old by their whitish centre, grayish translucent periphery, thin, dry appearance, and, under the microscope, the light brownish centre, the coarsely granular periphery (the granules appearing rather more as short lines than as granules) and slightly wavy or irregular contours.

A guinea-pig inoculated subcutaneously with 0.7 cc. of a suspension in sterilized NaCl solution from a pure culture of the diphtheritic bacillus from this case, died in 60–70 hours with exten-

sive local oedema, slight swelling of spleen, irregularly mottled kidneys, swollen and reddened supra-renal capsules, and moderate increase of pericardial and pleural fluids. The diphtheritic bacilli were obtained in pure culture from the oedematous fluid, but tubes inoculated from the lymph-glands, blood, spleen, liver and kidney remained sterile.

As the results and methods in subsequent cases are similar, they can be described more briefly.

CASE 2.—Negro woman, 18 years old, living in a family in which one child had recently died of diphtheria and four others are at present either sick or recovering from the disease, is said to have been sick about ten days, has a thick, rather tough, coherent, whitish membrane on uvula and soft palate. Patient died.

Cover-glass specimens showed, with the exception of very few streptococci and still fewer large cocci in clumps, only the Löffler bacillus, and this in large number, so that the specimens look almost like pure cultures.

Glycerine agar plates showed only in No. 1 very few streptococcus colonies, and large white colonies of a coarse coccus. The rest were colonies of the diphtheritic bacillus which appeared in pure culture in the numerous colonies of No. 2 and the scattered colonies of No. 3. Guinea-pig died with characteristic lesions in 48 hours.

CASE 3.—Mulatto boy, 4 years old. Three children in same family had been attacked with diphtheria and one had died. Grayish-white membrane on both tonsils and pharynx. Patient died.

Cover-glass specimens showed the Löffler bacillus as the predominant organism in large number. No streptococci found.

Cultures in glycerine agar were almost pure of the diphtheritic bacillus, the colonies being very abundant. No streptococcus colonies could be found. Guinea-pig died in 36 hours with characteristic lesions.

CASE 4.—Mulatto girl, 3 years old. Tonsils and posterior wall of pharynx covered with yellowish-gray diphtheritic membrane. Patient recovered.

Cover-glass preparations showed a large number of Löffler bacilli, mostly with swollen ends, sometimes of fusiform shape, also many short oval or slightly pointed bacilli and a few cocci, but none were found in chains.

Cultures in glycerine-agar presented, in about equal number, colonies of the Löffler bacillus and of a short oval bacillus. A few colonies of a coccus, occurring in short chains of five or six cocci, were found. Guinea-pig died with the characteristic lesions in 50 to 60 hours.

CASE 5.—Mulatto girl, 7 years old, in same family as preceding case. Firmly adherent tenacious gray membrane on left tonsil. Patient recovered.

Cover-glass specimen showed a large number of characteristic Löffler bacilli with their so-called involution forms, also many short oval bacilli, sometimes in clumps, a few coarse long bacilli, and a few large cocci. No streptococci.

The cultures and inoculation of guinea-pig gave the same results as in the preceding case.

CASE 6.—Mulatto boy, 6 years old, in same family as case 3. Thin gray adherent membrane covered both tonsils and was present also on pharynx. Patient died.

Cover-glass preparations showed as the predominant organism the Löffler bacillus in the form often of slightly curved club-shaped bacilli, frequently in clumps. In addition, short oval bacilli and cocci were present in considerable number.

The glycerine-agar plate cultures showed a large number of colonies of the Löffler bacillus, and a nearly equal number of colonies of the staphylococcus pyogenes aureus. A few streptococcus colonies were found and a large number of colonies of a short oval bacillus, occurring frequently in long chains. A guinea-pig died in 36 to 48 hours with the usual lesions.

CASE 7.—Girl, 7 years old. Sick for 3 days. Both tonsils covered with grayish-yellow diphtheritic membrane. Patient recovered.

Cover glass preparations showed Löffler bacilli, but not in so large number as various other organisms of different shapes including long thin rods and threads, short oval bacilli, large cocci, sarcinæ, etc.

Glycerine-agar and blood-serum cultures showed a moderate number of characteristic colonies, on the latter medium impure, of Löffler's bacilli and a larger number of colonies of various kinds of bacteria, chiefly streptococci, short oval bacilli and yeast fungi. A guinea-pig died in about 72 hours with characteristic lesions.

CASE 8.—Boy, 3 years old. Has been sick 4 days with fever and symptoms of croup. Examination of throat difficult on account of struggles of patient, but certainly no pseudo-membrane is visible on the tonsils or pharynx, but the attending physician thinks that he has detected it in the larynx. The breathing of the child indicates laryngeal obstruction and the cough is croupal in character. Notwithstanding repeated observations no false membrane could be detected at any time in pharynx or on tonsils or soft palate. Some muco-pus was scraped from the back of the pharynx and used for cultures. Patient recovered, improvement beginning in 5 days after onset of symptoms.

Blood-serum cultures showed a few large white colonies of a coarse coccus and a very large number of colonies of the Löffler bacillus, the latter representing almost a pure culture. A guinea-pig died after inoculation on the third day with characteristic lesions.

The preceding eight cases are all of the cases of diphtheria which we have had an opportunity of examining by culture methods. All were cases of primary diphtheria in which, with the possible exception of case 8, no doubt existed as to the clinical diagnosis of diphtheria. In case 8, the membranous deposit was limited to the larynx and trachea, the case being therefore one of primary laryngo-tracheal diphtheria, or so-called membranous croup, but in this case we obtained a nearly pure culture of the Löffler bacillus from the pharynx. The cases developed in patients living in widely separate parts of the city. In every case we found the Löffler bacillus and in most cases both on cover-slip preparations and in cultures this organism was the predominant one present, existing in the bits of membrane examined sometimes in nearly pure culture.

We have also made bacteriological examinations in a considerable number of cases which have served as a control for the cases of diphtheria. A few of these control cases have been children without any affection of the throat, most of them have been cases of simple inflammation of the tonsils or pharynx in which no suspicion of diphtheria existed, four have been cases of somewhat suspicious whitish exudation upon the tonsils, so-called follicular tonsillitis, in which at first the possibility of the existence of diphtheria has been entertained by the attending physician, but the subsequent history of the case has shown that it was not diphtheria. In one case we have made a bacteriological examina-

tion of the pharyngeal secretion three weeks after the diphtheritic membrane had disappeared. In none of these cases have we found the Löffler bacillus, nor any bacillus which an experienced bacteriologist would be likely to confound with the Löffler bacillus. We have not met with the so-called pseudo-diphtheritic bacillus, but in view of the limited number and special character of the cases which we have studied, we are not prepared to draw any conclusions as to the prevalence of the latter species.

The results of the bacteriological examination in the cases where at first there was some suspicion of diphtheria which the subsequent course showed to be unfounded, have convinced us as to the great practical value for diagnostic purposes of a bacteriological examination by cover glass specimens and by cultures in this large group of cases often so puzzling to the practitioner.

The following case is an illustration.

A boy 8 years old was brought to the dispensary of the Johns Hopkins Hospital. Nearly the whole of the right tonsil was covered with a soft yellowish-white membranous mass which could be easily removed with the forceps. The boy had fever but did not appear very sick. Cover-slip preparations showed a variety of micro-organisms, the most conspicuous ones being cocci both in groups and in chains, rather short thick rods, and spirochaete. The cultures showed as the predominant organism colonies of streptococci and of staphylococcus pyogenes aureus. Notwithstanding extended search not a single colony of the Löffler bacillus was found. In three days the inflammation had disappeared and the boy was well. None of several children living in the same apartments developed any soreness of the throat. We have examined with similar results three other analogous cases.

We have had the opportunity of examining microscopically two large pieces of diphtheritic membrane which had been expelled by patients living at a distance and which hardened in alcohol were sent to the laboratory. Sections of these membranes after staining by Gram's method showed in clumps and singly the characteristic Löffler bacilli which could be readily identified by their peculiar shapes and staining.

The only species of bacteria which we have found constantly in the cases of diphtheria has been the Löffler bacillus. Two other species have been present in many cases, viz: the well-known streptococcus, which grows in much smaller colonies and less rapidly than the Löffler bacillus and a short oval, often

slightly pointed bacillus, growing in long chains running parallel to each other. There are often marked irregularities in shape and especially in size of this bacillus, even of individuals in the same chain. The colonies of this organism are grayish-white, moist, larger than those of the streptococcus but smaller than those of the Löffler bacillus. The colonies of this short bacterium bear more resemblance to those of the Löffler bacillus than any others which we have met with, although colonies of other organisms bear a greater similarity in rapidity of growth and in size, notably those of a large coccus, but they are readily distinguished by a more opaque white appearance.

We have not utilized any of the material from the cases of diphtheria which we examined by culture methods, in order to study the distribution of the bacteria in the membranes, as the material rarely sufficed for hardening and making sections. We have, however, studied sections prepared by Dr. Councilman from a large number of cases of diphtheria. These were of cases of diphtheria dying in Frankfurt, Germany, the sections being made under Prof. Weigert's direction, and stained by his new modification of the fibrin stain, the details of which have not yet been published. Upon these sections both the fibrin and the Löffler bacilli are brilliantly stained, and the diphtheritic foci described by Oertel in the mucous membrane and in lymphatic glands and elsewhere are well shown. The Löffler bacilli, with their irregular, often bent, shapes, segmented appearances and deeply stained granules, are clearly to be seen. The usual arrangement is as follows: Most superficially is a deeply stained mass containing various kinds of bacteria, among which the Löffler bacilli are to be recognized; beneath this comes a feebly stained layer, rich in cells, but containing little or no fibrin; in this layer the Löffler bacilli, both singly and in clumps, are much more readily made out. In the deeper parts of this pale layer, the Löffler bacilli are often the only bacteria present, and especially just above the deeply stained fibrin layer, characteristic clumps of these bacilli are present. Streptococci may, however, also be present in this layer. The layer resting upon the mucous membrane is rich in fibrin and therefore stains deeply with the Weigert stain. In this fibrin layer, except in its most superficial parts, no Löffler bacilli are to be seen. This arrangement of the bacilli corresponds with the description given by Klebs and by Löffler. We have never found the bacilli in the mucous membrane affected nor in sections from internal organs,

not even in the diphtheritic foci presenting nuclear fragmentation and hyaline masses and fibrin. The Löffler bacilli, therefore, not only do not invade the affected mucous membrane; they do not usually even extend throughout the entire thickness of the diphtheritic pseudo-membrane. On the other hand, various complications of diphtheria may be due to the secondary invasion of other organisms, notably streptococci, which are occasionally found in the mucous membrane, lymphatic glands and elsewhere.

Our studies of the morphological and biological properties of the Klebs-Löffler bacillus are, in the main, simply confirmatory of the results of other investigators, and we shall therefore give only a brief description of this part of our work, emphasizing only certain points which are not clearly brought out in the publications of previous observers.

The bacillus is non-motile. It varies greatly in size and shape, averaging 2.5 to 3 μ in length and 0.5 to 0.8 μ in thickness. Its morphological characters are so peculiar as to render its identification on cover-slip preparations and on sections from diphtheritic membranes in most cases an easy matter. Sometimes, appearing as a regular straight or slightly bent rod, with rounded ends, it is especially characteristic to find irregular and often bizarre forms, such as rods with one or both ends swollen, and very frequently rods broken at irregular intervals into short sharply-marked segments, either round, oval or with straight sides. Some forms stain uniformly, others in various irregular ways, the most common being the appearance of deeply stained granules in a lightly stained bacillus.

Most writers are silent upon the question as to whether the Löffler bacillus stains by Gram's method or not. It is stated by Beck¹ that in sections it cannot be stained either by Gram's or by Weigert's fibrin method. This is contrary to our experience. We find that both in sections and in cover-glass specimens from blood-serum cultures, the bacilli stain well by Gram's method. Only by prolonged decolorization of cover-glass preparations with alcohol and oil of cloves do most of the bacilli lose their color. We find both Gram's and Weigert's fibrin method admirably adapted for the staining of sections of diphtheritic membranes in order to demonstrate the Löffler bacilli. Nothing can surpass in brilliancy and sharp differentiation, sections stained doubly by the modified Weigert's fibrin stain and picro-carmin.

¹ Beck, *Zeitschrift für Hygiene*, Bd. VIII, p. 436.

The Löffler bacillus can be cultivated readily upon all of the ordinary culture media, viz.: alkaline bouillon, nutrient gelatine, nutrient agar, glycerine agar, blood-serum and potato. The medium upon which it grows most rapidly and luxuriantly, and which is best adapted for determining its presence in diphtheritic exudations, is the mixture of blood-serum and bouillon as recommended by Löffler (three parts blood-serum and one part bouillon, containing 1 per cent. peptone, 1 per cent. grape sugar and 0.5 per cent. chloride of sodium; the mixture, of course, to be solidified in the usual way for blood-serum). For blood-serum can be substituted serous transudations, but these have not seemed to us quite equal to the blood-serum. On the blood-serum mixture the colonies of the Löffler bacillus grow so much more rapidly than other organisms usually present in the secretions and exudations in the throat that at the end of twenty-four hours they are often the only colonies which attract attention, and if other colonies of similar size are present they are generally of quite different aspect. A blood-serum tube, studded over with coalescent or scattered colonies of the Löffler bacillus, is so characteristic in appearance that one can anticipate with tolerable certainty the result of the microscopical examination. By this method of cultivation a diagnosis can usually be made on the day following the inoculation of the tube. The colonies are large, round, elevated, grayish-white, with a centre more opaque than the slightly irregular periphery. The surface of the colony is at first moist, but after a day or two rather dry in appearance.

The appearances of the young colonies in glycerine agar and plain nutrient agar are well depicted by Flügge.² We have found the bacillus to grow well in agar, as a rule, but often the first inoculations of a plain nutrient agar tube have given comparatively feeble growths while subsequent inoculations have afforded more abundant growths. Nutrient gelatine, of a feebly alkaline reaction, we have found an excellent medium at a temperature of 22° C., and we have repeatedly obtained pure colonies of the Löffler bacillus on this medium from diphtheritic deposits. Like Fränkel and Brieger,³ we have sometimes observed a diffusely cloudy growth of the Löffler bacillus in alkaline bouillon, but, as a rule, the growth has been, as Löffler described it, in the form of small whitish specks at the bottom and along the sides of the tube,

² Flügge, *Die Mikroorganismen*, p. 229. Leipzig, 1886.

³ Fränkel and Brieger, *Berliner klin. Wochenschrift*, 1890, p. 248.

leaving the fluid clear. In bouillon cultures, standing a long time in the thermostat at 35° C., sometimes a soft whitish membrane forms over a part of the surface.¹ The reaction becomes at first acid and subsequently again alkaline, changes which can be well observed in cultivations in bouillon to which a little rosolic acid has been added.

Löffler and following him most writers state that the diphtheritic bacillus does not grow on potato unless it is rendered alkaline. Zarniko,² however, says that after 8 to 10 days, at a temperature of 35° C., a growth of extremely distorted rods can be recognized as a delicate whitish deposit where the potato has lost its glistening appearance. Our experience has been that the bacillus diphtheriæ grows on ordinary steamed potato without any preliminary treatment, but that the growth is usually entirely invisible or is indicated by a dry thin glaze after several days. Doubtless the invisible character of the growth has led most observers into the error of supposing that no growth existed, whereas the microscopical examination reveals a tolerably abundant growth, which on the first potato is often feebler than on succeeding ones. Irregular forms are particularly numerous in potato cultures, and in general the rods are thicker than on other media. In 24 hours, at a temperature of 35° C., microscopical examination shows distinct growth. We have cultivated the bacillus for many generations on potato.

We have tested the thermal death point upon bouillon cultures and suspensions in sterilized salt solution in capillary glass tubes and have found that the bacillus is killed at a temperature of 58° C. in ten minutes. It does not form spores.

We have used guinea-pigs, rabbits and kittens for inoculation experiments with pure cultures.

Most of our inoculations have been made upon guinea-pigs by subcutaneous injections of 0.1 to 0.7 cc. bouillon cultures or dilute suspensions in sterilized salt solution of the Löffler bacillus or by inserting a platinum needle carrying a small quantity of the culture. The duration of life in these animals has varied from 24 hours to four to five days. The usual changes have been an extensive local oedema with more or less hyperaemia and ecchymosis at the site of inoculation, frequently swollen and reddened lymphatic glands, increased serous fluid in the peritoneum, pleura

¹ Noticed also by Roux and Yersin, *Annales de l'Institut Pasteur*, 1889, p. 274.

² Zarniko, *Centralblatt f. Bakteriologie*, Bd. VI, p. 180.

and pericardium, enlarged and hemorrhagic supra-renal capsules, occasionally slightly swollen spleen, sometimes fatty degenerations in the liver, kidney and myocardium. There are variations as to the extent and presence of these lesions in different cases which need not be here described. We have always found the Löffler bacilli at the seat of inoculation, most abundant in a grayish-white fibrino-purulent exudate present at the point of inoculation and becoming fewer at a distance from this, so that the more remote parts of the oedematous fluid do not contain any bacilli. We find the bacilli not only free but contained in large number in leucocytes, some of which have fragmented nuclei or have lost their nuclei. The bacilli within the leucocytes as well as some outside sometimes stain very faintly and irregularly and may appear disintegrated and dead. We have never failed to obtain the bacilli in cultures from the point of inoculation and think it likely that the negative results of some observers in demonstrating the bacilli are due to making their examinations with the oedematous fluid at a place too remote from the point of inoculation. In all cases culture tubes inoculated with the blood, spleen, liver, kidney, supra-renal capsules, lymphatic glands and serous transudates, have yielded negative results.

Rabbits are less susceptible, but they have usually died in the course of 5 to 20 days after inoculation of even small quantities. They develop as a rule paralysis affecting the posterior extremities, and we have also observed a peculiar tremulous motion and irregular respiration.

An affection may be reproduced by inoculation of certain animals in all respect identical with the disease diphtheria as it exists in man. The following case selected from several similar ones is an example.

A half grown kitten is inoculated into the trachea with one platinum loop from a pure culture of the Löffler bacillus on glycerine-agar, 11 days old, derived from Case 4. For the inoculation a small median incision was made over the trachea in which a hole just large enough to admit the platinum loop was made. The culture was rubbed over the mucosa of the trachea for an extent about 3 ctm. in length, and in this process sufficient force was used to abrade the mucous membrane. On the day following the inoculation no special alteration in the animal was observed, but on the morning of the second day it was found very

weak. In the course of this day it became so weak as to lie completely motionless, apparently unconscious with very feeble shallow respiration. Several times it was thought to be dead but on careful examination proved still to be breathing feebly. It was found dead on the morning of the 3rd day. At the autopsy the wound was found gaping and covered with a grayish, adherent, necrotic, distinctly diphtheritic layer. For a considerable distance around the wound the subcutaneous tissues were very oedematous, the oedema extending from the lower jaw down over the sternum and to the sides of the neck and along the anterior extremities. The lymphatic glands at the angle of the jaw were markedly swollen and reddened. The mucous membrane of the trachea beginning at the larynx and extending down for 6 cm. was covered with a tolerably firm, grayish white, loosely attached pseudo-membrane in all respects identical with the croupous membranes observed in the same situation in cases of human diphtheria. No other noticeable lesion was found in the animal unless it be a greater degree of fatty metamorphosis of the renal epithelium than is normal in kittens. Both in the pseudo-membrane and in the oedematous fluid adjacent to the wound many bacilli diphtheriae, occurring often in clumps, were recognized on cover-slip preparations and this species grew in a large number of colonies on plate cultures. It is interesting to note that the faithful attendants of the Löffler bacillus, streptococci, were present also in the same situations.

The mere production of a pseudo-membranous inflammation can not be claimed as establishing by itself alone the etiological role of the bacillus diphtheriae as such an inflammation as is well known can be produced experimentally in a variety of ways. Greater significance attaches to the demonstration of certain peculiar alterations no less characteristic of the specific action of the diphtheritic virus. As in human diphtheria so in all susceptible animals inoculated with the Löffler bacillus, there is only a local development of the bacilli at the point of infection, and it has been demonstrated especially by Roux and Yersin, and by Brieger and Fränkel¹ that extraordinarily poisonous products formed by the bacilli are absorbed from the site of local development of the bacilli and give rise to the constitutional symptoms and the lesions of internal organs. The separation of these

¹ Roux and Yersin, *Annales de l'Institut Pasteur*, 1888, 1889, 1890. Brieger and Fränkel, *Berliner Klinische Wochenschrift*, 1890.

products in a condition approaching purity in the form of one or more toxic albuminous substances and the production by injecting them of paralyzes and of other symptoms and lesions (save the pseudo-membrane) of diphtheria are unquestionably brilliant achievements, and have added greatly to the chain of proof leading to the recognition of the Löffler bacillus as the specific cause of diphtheria. Our studies so far as they have been directed to this part of the subject are confirmatory of the results reported by Roux and Yersin and by Brieger and Fränkel.

We have directed our attention with especial interest to the question as to whether the interesting and important discoveries of Oertel,¹ relating to the special action of the diphtheritic virus upon cells and the appearance of peculiar foci of nuclear fragmentation and cell-death with other changes in the lymphatic glands, and the spleen could be confirmed by experiments upon animals. Babes² has also communicated some observations upon this point. Dr. Flexner is at present engaged in the Pathological Laboratory in the examination of this question, and the results will be reported subsequently. Here we will only mention that, in a number of instances, we have been able to show not only extensive cell-death characterized by an extreme degree of fragmentation of nuclei at the site of inoculation, but also similar changes in the spleen, lymphatic glands and elsewhere. These peculiar alterations, as Oertel has shown, in their distribution, are characteristic of human diphtheria, and the demonstration of similar changes in animals, inoculated with the Löffler bacillus, is no small additional proof that diphtheria is caused by this micro-organism.

It would seem, therefore, that every postulate necessary to prove that the specific cause of primary diphtheria is the Klebs-Löffler bacillus has been fulfilled, and that this bacillus may be designated the bacillus diphtheriæ. This bacillus is present in every case of primary diphtheria, in such number and situation as to explain the local manifestations of the disease, it can be readily isolated in pure culture, and a disease identical in all respects with human diphtheria can be produced experimentally by the inoculation of pure cultures. The investigation of the toxic albumens, produced by this remarkable bacillus, has cleared up many obscure points.

The only points which can be brought forward as tending to cast any doubt upon the recognition of this bacillus, as the spe-

¹ Oertel, *Die Pathogenese der epidemischen Diphtherie nach ihrer histologischen Begründung*, Leipzig, 1888.

² Babes, *Centralblatt f. Bakteriologie*, Bd. VIII, p. 741.

cific cause of diphtheria, are the very exceptional observation of an apparently identical bacillus in the throat of healthy children and the more frequent observation of a bacillus, usually called the pseudo-diphtheritic bacillus, which differs from the true bacillus diphtheriæ chiefly or only by the absence of pathogenic properties when inoculated into animals.

The finding in healthy individuals of a bacillus apparently identical with the bacillus diphtheriæ is such a rare occurrence that Löffler, who made the first observation of this nature, has not been able to repeat it during several years of search. When found it has been present in such small number as to present a marked contrast to the results in cases of diphtheria. The exceptional occurrence in healthy persons of the bacillus diphtheriæ in such small number as to indicate that it does not find suitable conditions for its multiplication and injurious action upon the tissues, seems to us in no way opposed to the acceptance of this bacillus as the specific cause of diphtheria. Experiments upon susceptible animals show that to produce in them a pseudo-membranous inflammation of a mucous membrane by inoculation with the diphtheritic bacillus, it does not suffice simply to apply the bacilli to the surface of the mucous membrane but that an actual injury must be done to the membrane. While it is by no means proven that an injury to or a morbid condition of the mucous membrane is necessary for the infection of human beings with the diphtheritic virus, nevertheless such conditions are recognized as important predisposing causes, and clinical observations point to differences in susceptibility to infection with this virus, so that there is no reason why in some healthy persons the bacillus diphtheriæ might not be present in the mouth without doing any damage.

The status of the so-called pseudo-diphtheritic bacillus can not be considered as settled. Whereas Löffler finds such morphological and biological differences between it and the true bacillus diphtheriæ as to lead him to regard it as unquestionably a different species, Roux and Yersin consider that it is simply a modification of the bacillus diphtheriæ, from which it differs only by absence of virulence. They claim that the pseudo-diphtheritic bacillus is identical in its morphology and its behavior on culture media with the diphtheritic bacillus, and, calling attention to the marked variations in virulence of the diphtheritic bacilli found not only in different cases but in the same case of diphtheria, they assert that every

transition exists between the most virulent diphtheritic bacillus and the non-virulent pseudo-diphtheritic bacillus. Although their observations and experiments have not proven their belief, they bring forward strong arguments in support of the view that the bacillus diphtheriæ under certain circumstances may be converted into a non-virulent variety and conversely the non-virulent bacillus into the virulent form. The pseudo-diphtheritic bacillus appears to be not particularly uncommon in the mouth and throat of healthy children or of children not affected with diphtheria, but when present it does not occur in anything approaching the number of diphtheritic bacilli ordinarily found in cases of diphtheria, so that a diagnostic error is not likely to result even in cases in which it exists. The inoculation of animals will settle the question. Whatever may prove to be the nature of the pseudo-diphtheritic bacillus, it does not seem to us that the observation of a non-virulent bacillus resembling or even identical morphologically and in modes of growth on culture media with the bacillus diphtheriæ can be justly urged as an argument opposed to the recognition of the latter organism as the specific cause of diphtheria, and indeed certain facts in the development of diphtheria may possibly find their best explanation in the assumption that the bacillus diphtheriæ may exist in a non-virulent state, and under certain conditions which we do not now understand may acquire virulence.

There are few infectious diseases upon which greater light has been shed by the discovery and study of their specific micro-organisms than has been the case with diphtheria.

We are now in possession of a positive means of diagnosis, and while it may be questioned whether practitioners of medicine in general are likely to be in a position to make use of this means, the method is not difficult and can be readily applied. We have already learned that there are pseudo-membranous anginas which must be separated etiologically from true diphtheria, and also that diphtheria may exist in extremely mild forms even without visible pseudo-membranous deposits. We are now in a position to clear up a hitherto confusing and much disputed chapter in etiology. The endless controversy as to whether diphtheria is primarily a local or a general disease is settled in favor of the doctrine that it is primarily local, and that the grave constitutional symptoms are the result of intoxication with poisonous products, formed by the local action of the bacilli. We are able to study experimentally the various effects resulting from the action upon

the animal body of the specific toxic products of the diphtheritic germ. We can separate the alterations belonging to the disease itself from the many complications of the disease. Intelligent measures of prophylaxis can be based upon a definite knowledge of the characters of the specific germ and its behavior in the body, and such measures have already been formulated. Rational indications for treatment can be established.

That the blood-serum of animals rendered artificially immune against the diphtheritic bacillus and its products is capable of destroying or nullifying the effects of the toxic albumens of diphtheria, although not of killing the bacilli, is a new conception in medicine, and this, together with other observations of Fränkel and of Behring upon the production of immunity, opens up new vistas which may lead to results of the greatest scientific and practical importance.

